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**Delineating the phenotypic spectrum of Bainbridge-Ropers syndrome: Twelve new patients with de novo, heterozygous, loss-of-function mutations in ASXL3 and review of published literature**

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**Short Title:** Bainbridge-Ropers syndrome: twelve new cases and literature review

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## **KEYWORDS:**

ASXL3, intellectual disability, Marfanoid habitus, heterozygous, loss-of-function

## **ABSTRACT**

**Background:** Bainbridge–Ropers syndrome (BRPS) is a recently described developmental disorder caused by de novo truncating mutations in the Additional sex combs-like 3 (ASXL3) gene. To date there have been fewer than ten reported patients.

**Objectives:** Here we delineate the BRPS phenotype further by describing a series of twelve previously unreported patients identified by the Deciphering Developmental Disorders (DDD) study.

**Methods:** Trio-based exome sequencing was performed on all twelve patients included in this study which found a de novo truncating mutation in ASXL3. Detailed phenotypic information and patient images were collected and summarised as part of this study.

**Results:** By obtaining genotype: phenotype data, we have been able to demonstrate a second mutations cluster region within ASXL3. This report expands the phenotype of older patients with BRPS; common emerging features include severe intellectual disability (12/12), poor/absent speech (12/12), autistic traits (9/12), distinct face (arched eyebrows, prominent

forehead, high-arched palate, hypertelorism and down-slanting palpebral fissures), (9/12), hypotonia (12/12) and significant feeding difficulties (12) when young.

**Discussion:** Similarities in the patients reported previously in comparison to this cohort included their distinctive cranio-facial features, feeding problems, absent/limited speech, and intellectual disability. Shared behavioural phenotypes include autistic traits, hand-flapping, rocking, aggressive behaviour, and sleep disturbance.

**Conclusions:** This series expands the phenotypic spectrum of this severe disorder and highlights its surprisingly high frequency. With the advent of advanced genomic screening, we are likely to identify more variants in this gene presenting with a variable phenotype which this study will explore.

## INTRODUCTION

Large scale whole exome sequencing projects such as the Deciphering Developmental Disorders (DDD) Project have led to the discovery of a number of new genes underlying developmental disorders [1,2]. A reverse-genetics approach has proven particularly important for the discovery of disorders like Bainbridge–Ropers syndrome (BRPS), whose main clinical features are non-specific, especially when looked at in isolation or with a small number of patients. De novo mutation status is the first clue to potential pathogenicity of a given variant and such mutations are known to constitute a significant proportion of the underlying causes of moderate and severe intellectual disability (ID) [3].

ASXL1, ASXL2 and ASXL3 are human homologs of the *Drosophila* additional sex combs (*asx*) gene that encode putative polycomb proteins and are likely to act as histone

methyltransferases in complexes with other proteins [4]. Polycomb group proteins are implicated in embryogenesis and carcinogenesis through transcriptional regulation of target genes; the ASXL1 gene is thought to be one of the most frequently mutated genes in malignant myeloid diseases; ASXL is a scaffold protein interacting with methyltransferases and additional proteins of the epigenetic machinery [5,6]. Truncating mutations in ASXL1 have been reported in association with Bohring-Opitz syndrome (BOS) which has phenotypic overlap with BRPS [7]. More recently, truncating mutations in ASXL2 were reported in association with a newly recognisable clinical phenotype [8].

Srivastava et al., 2016 showed that ASXL3 interacts with BAP1, a hydrolase that removes mono-ubiquitin from histone H2A lysine 119 (H2AK119Ub1) as a component of the Polycomb repressive deubiquitination (PR-DUB) complex [9]. The authors observed a significant increase in H2AK119Ub1 in ASXL3 patient fibroblasts, highlighting an important functional role for ASXL3 in PR-DUB mediated deubiquitination. Transcriptome analysis revealed >500 genes differentially expressed in ASXL3 patient fibroblasts relative to controls, and these genes were enriched for those involved with molecular processes impacting transcriptional regulation, development and proliferation.

ASXL3 is expressed in similar tissues to ASXL1 including brain, spinal cord, kidney, liver, and bone marrow, but at a lower level [10]. The high correlation of expression patterns between ASXL1 and ASXL3 may account for some of the shared phenotypic features.

Heterozygous, de novo loss-of-function mutations in ASXL3, underlying the Bainbridge–Ropers syndrome (BRPS: OMIM #615485) have been described in 9 individuals to date [9, 11-13]. The major phenotypic features described in the majority of patients so far include

failure to thrive, global developmental delay, feeding problems, hypotonia, dysmorphic features, profound speech delay and intellectual disability. Here we present genetic and phenotypic information on 12 previously unreported individuals with de novo truncating mutations in *ASXL3*, all of which were detected via the trio exome sequencing carried out by the DDD Project. Additional clinical features of BRPS are likely to emerge with identification of additional patients through such large scale exome sequencing projects as described here.

## **METHODS**

### **EXOME SEQUENCING**

In all twelve individuals identified via the DDD study, trio-based exome sequencing was performed on the affected individual and their parents, as previously described by Wright et al., 2014. Each affected individual has also had a high-resolution analysis for copy number abnormalities using array-based comparative genomic hybridization (aCGH). Putative de novo mutations were identified from exome data using DeNovoGear software as described by Ramu et al., 2013 and were validated using targeted Sanger sequencing [14,15].

All recruited patients had the following de novo heterozygous pathogenic mutations identified which confirmed the diagnosis of Bainbridge-Ropers syndrome (OMIM: 615485):

Patient 1: c.4330C>T, p.(Arg1444\*)

Patient 2: c.1201del, p.(Ala401GlnfsTer8)

Patient 3: c.1074T>A, p.(Tyr358\*)

Patient 4: c.4144C>T, p.(Gln1382\*)

Patient 5: c.1783C>T, p.(Gln595\*)

Patient 6: c.3355dup, p.(His1119Profs\*7)

Patient 7: c.1082dup, p.(Leu362AlafsTer23)

Patient 8: c.3635T>G, p.(Leu1212\*)

Patient 9: c. 3127\_3128dup, p.(Gly1045Valfs\*99)

Patient 10: c.3178dup, p.(Arg1060Profs\*50)

Patient 11: c.1484insTGAA, p.(Asp497\*)

Patient 12: c.1491dup, p.(Asn498\*)

Mutation nomenclature is according to HGVS recommendations (<http://varnomen.hgvs.org/>), and is based on reference transcript NM\_030632.2.

## **PATIENT ASCERTAINMENT**

All twelve individuals were recruited via UK NHS Regional Genetics Services onto the Deciphering Developmental Disorders (DDD) Project ([www.ddduk.org](http://www.ddduk.org)). As part of that study, patient and parental samples receive array CGH and exome sequencing analysis and findings of potential clinical significance are reported back to recruiting clinical geneticists. Any significant findings are usually validated by an accredited UK NHS diagnostic genetics laboratory before being reported to patients and their families; mutations described in this paper have been validated as such. Patient phenotype information was provided to the authors via Clinical Geneticists from several UK NHS genetics services. See Table 1 for a summary of the clinical and molecular findings.

## **RESULTS**

### **ASXL3 MUTATIONS**

Previously reported truncating *ASXL3* mutations cluster mainly within the 5' end of exon 11 between codons 404 and 659. This region lies in-between the N-terminal protein scaffolding functional domains of the gene and the C-terminal chromatin/DNA-targeting functional domain. Srivastava et al., 2016 reported two mutations significantly 3' to this main cluster region, at codons 1122 and 1444 [9]. One of the patients (Patient 1) within our cohort carries the same c.4330C>T p.(Arg1444\*) mutation as the patient reported by Srivastava et al, 2016 suggesting it as a possible recurrent mutation.

Among our cohort, 5/12 (Patients 2, 3, 7, 11, 12) of the mutations could be described as occurring within the originally reported mutation cluster region, 1/12 (Patient 5); c.1783C>T, p.(Gln595\*), maps more 3', and the remaining 6 (Patients 1, 4, 6, 8, 9, 10) lie further downstream within the more 3' region, reported by Srivastava et al, 2016, which could be considered as a distinct mutation cluster region, extending between codons 1045 and 1444 [9]. All these mutations are publicly available on [www.ddduk.org](http://www.ddduk.org).

## PATIENT PHENOTYPES

**Antenatal history and birth:** Polyhydramnios and concerns regarding poor growth were noted in 1/12 but otherwise unremarkable. For 9/12 patients, a caesarean section was performed, mostly due to breech presentation. All 12 patients had an average birth weight and apart from 4/12 patients who were admitted to the Neonatal unit for respiratory difficulties/apnoea, the remainder neonatal period was uneventful.

**Feeding problems:** Consistent with previous reports, 9/12 patients were reported to have significant feeding problems, often including gastro-oesophageal reflux, requiring



intervention in the form of nasogastric tube feeding, fundoplication. The majority were described as having failure to gain weight with poor appetite.

**Growth:** Patients reported here had consistent poor growth with weight and height below the 0.4<sup>th</sup> centile and relative microcephaly (7/12). This is in keeping with previously reported literature.

**Craniofacial features:** 9/12 have a high-arched palate, distinctive facial dysmorphism as described below (Figure 1).

**Dysmorphic features:** 10/12 had down-slanting palpebral fissures and 2 had up-slanting palpebral fissures; both have previously been reported but down-slanting seems to be more common. A long, tubular nose with a prominent nasal bridge is apparent in most. Most of the individuals have a broad nasal tip with low columella. The mouth is wide with full (everted) lower lip. Hypertelorism, a narrow head shape with prominent forehead, 'pencilled' and/or high-arched eyebrows and crowded teeth were also common features.

**Other significant features:** 12/12 had significant hypotonia, 7/12 had strabismus of varying severity. 3 patients had seizures, previously reported in 2 other ASXL3 patients. Patient 2 had scoliosis requiring surgery. 3 patients had arachnodactyly, not previously reported in any ASXL3 patients. 3/12 appears to have a Marfanoid habitus with arachnodactyly, tall stature, pes planus and scoliosis.

**Intellectual Disability:** The level of intellectual disability ranged from moderate to profound but more likely at the severe end of the spectrum. All patients had ID of varying degree;

generally severe. Most were very delayed in walking unassisted and 2 remained entirely non-ambulant. 9/12 patients were entirely non-verbal, including Patient 2 at 22 years of age. 9/12 patients had either formally diagnosed autism or autism spectrum disorder, or were described as having autistic features. 3 patients exhibited hand-flapping, rocking. All the patients were in a special needs school requiring significant help.

**Relevant negative findings:** Seizures does not appear to be a major feature, seen in only 3/12 patients and generally well-controlled absence seizures; MRI-brain imaging only showed non-specific features with white matter changes (3/12) and vermis hypoplasia (1/12) which is relevant given the significance of intellectual disability in this cohort of patients.

## DISCUSSION

The recently described Bainbridge–Ropers syndrome (BRS; OMIM # 615485), associated with de novo truncating mutations in the Additional sex combs-like 3 (ASXL3) gene (OMIM \* 615115), shows phenotypic overlap with Bohring-Opitz syndrome, which is associated with de novo truncating mutations in ASXL1 (OMIM \* 612990). Bohring-Opitz syndrome (BOS; OMIM # 605039) is characterised by distinct craniofacial features and posture, severe intellectual disability, feeding problems, small size at birth, and failure to thrive.

Bainbridge et al. 2013 reported a series of four unrelated probands with de novo, heterozygous, truncating mutations in ASXL3, sharing similar phenotypes, including severe feeding difficulties, failure to thrive, and neurologic abnormalities with significant developmental delay [11]. More recently, truncating mutations in ASXL2 were reported as being associated with a newly recognisable syndrome with overlapping features to BOS and BRPS [8]. In this report, the authors described six unrelated patients with de novo truncating

mutations in ASXL2 with shared clinical features including intellectual disability, macrocephaly, distinct facies, facial nevi, feeding difficulties and hypotonia. Comparison of patients reported in this with BRPS shows the facial dysmorphism to be more similar to BOS with macrocephaly, arched eyebrows, synophrys and facial nevi rather than with BRPS. Other distinguishing features included macrocephaly, congenital heart disease, structural brain malformations and seizures in these patients, which differs to the BRPS cohort. However, there are emerging similarities within this group of conditions, including hypotonia, feeding difficulties and intellectual disability, which will become more apparent as more patients are reported with ASXL2 mutations.

To date there have been fewer than ten reported patients with de novo truncating ASXL3 mutations. Emerging similarities include, distinctive cranio-facial features with arched eyebrows, prominent forehead, high-arched palate, hypertelorism with down-slanting palpebral fissures; significant feeding difficulties needing support; profound/ severe intellectual disability; emerging behavioural phenotype consisting of autistic traits, hand-flapping, rocking, aggressive behaviour, sleep issues with absent/ poor speech. Table 1 provides a comprehensive summary of reported features in this cohort: predominant features in the phenotype are normal pregnancy, higher incidence of caesarean section due to breech presentation, relative microcephaly, significant feeding difficulties, facial dysmorphism, high-arched palate, strabismus, hypotonia, skeletal features including a Marfanoid habitus (especially in the older patients), severe intellectual disability with poor/ absent speech, autistic traits, need for special education. Seizures, structural malformations of internal organs including the brain, kidneys do not appear to be a predominant part of their phenotype. However, this is likely to be revised/ expanded as more patients are described with BRPS.

There is a wide age range (4-22 years), this being the first report of older patients with BRPS. The older patients in this cohort all have moderate to severe ID, autistic features, attended a special needs school and are in assisted living. Seizures are a component but not a predominant part of their phenotype and they do not appear to have any major structural associations with this diagnosis as they have grown older. The behavioural phenotype appears to be in keeping with other severe developmental disorders with absent/ poor speech, periods of agitation, frustration and poor sleep.

Though Bainbridge-Ropers syndrome (BRPS) is likely to remain a challenging syndrome to recognise clinically, this cohort of patients has enabled further delineation and expansion of the phenotype. Results of analysis of the first several thousand patient trios within the DDD Project suggests that de novo ASXL3 mutations are among the more common underlying causes of disease within the DDD cohort (at time of writing, ASXL3 ranks number 12 out of the top 20 genes in which a pathogenic de novo mutation has been found), and therefore it is expected that there will be many more BRPS patients diagnosed in the near future, further defining the associated clinical spectrum.

Our cohort has also firmly established a second, 3' mutational cluster region within ASXL3 which may be of significance to disease mechanism. In regards to this, an ASXL3 mRNA transcript carrying the c.1448dupT truncating mutation has previously been shown to be prone to nonsense-mediated decay, with resultant reduction in expression of ASXL3 [6]. Consistent with previous reports and consistent with this disease mechanism, the cohort of patients described here do not show a correlation between phenotypic features or severity and mutation position. It has previously been noted that several truncating mutations in ASXL3

are described in databases composed of sequence variants from phenotypically normal individuals (see Figure 2). To date, there are 4 such mutations within the ExAC dataset, each identified within only one individual within the dataset, and they occur both 5' to the original 5' mutational cluster region (MCR) and 3' to the new 3' cluster region, and also in between the two cluster regions. The explanation for these mutations is as yet uncertain.

We have also been able to collect phenotypic data from several patients with previously unreported missense variants in *ASXL3* (including p.Ser86Ala, p.Lys1026Asn, p.Arg933Trp and p.Ser720Cys). However, in each case the variants were inherited from clinically unaffected parents and the patients had a very dissimilar presentation in comparison to the clinical presentation associated with *ASXL3* loss-of-function mutations. Without further investigation it cannot be ruled out that these variants are of clinical significance, however it is unlikely that they are the sole cause of the phenotype observed in these patients. It is possible that they represent rare polymorphisms. In support of this, all of these variants are found, albeit at low frequency, within healthy control populations (Exome Aggregation Consortium, Cambridge, MA, URL: <http://exac.broadinstitute.org>).

Ropers et al., 2015 previously highlighted the presence of truncating mutations in *ASXL3* and several other dominant genes for intellectual disability or related disorders, within healthy control populations, suggesting the possibility of incomplete penetrance for truncating mutations within these genes [14]. This included *ASXL1*, for which 56 such mutations were found within the ExAC dataset.

Whilst it cannot be entirely ruled out that truncating *ASXL3* mutations exhibit incomplete penetrance, the number of such mutations (four) found within the ExAC data is still relatively

small compared to ASXL1 and the other genes examined, and the list of reported patients with ASXL3 truncating mutations that have a Bainbridge-Ropers syndrome consistent phenotype is growing.

Therefore, it seems reasonable that all four of these ASXL3 mutations may be accounted for by the various other explanations that Ropers put forward, for example, two of the mutations occur at the extreme 3' end of the gene, and may therefore escape nonsense mediated decay (NMD), retaining protein activity. Bainbridge et al., 2013 suggested that mutations may arise post-zygotically or during later embryogenesis and thus the phenotypic variability or incomplete penetrance may be explained by mosaicism [11]. With the increasingly wider access of high read-depth exome sequencing for genetic diagnosis of children with developmental disorders it seems likely that this question will eventually be answered as more data emerges.

## CONCLUSIONS

In this series, we report 12 patients with ASXL3 loss-of-function de novo variant and expand the phenotype of Bainbridge-Ropers syndrome. New specific associated clinical features have become apparent, such as hypotonia, Marfanoid habitus, and arachnodactyly. This cohort is consistent with previously reported patients with regards to the facial features and also confirms pertinent negative features such as lack of significant findings on brain imaging, and lack of seizures. This research further reiterates the power of whole exome studies in conjunction with a detailed clinical phenotype in providing an explanation for our patient's difficulties and a unifying diagnosis for their concerns.

## **STATEMENTS:**

### **A. Funding:**

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### **B. Acknowledgements:**

We would like to thank all these families for consenting to publication.

### **C. Contributorship Statement:**

All authors recruited their respective patients to the DDD study and provided data regarding their patients; DDD study provided trio exome sequencing data. MB and JB planned the study; MB recruited Patient 1 to DDD; wrote manuscript; all authors reviewed and contributed to the manuscript.

**D. Competing Interest:** None to declare for all authors.

## **FIGURE AND TABLE LEGENDS**

**Figure 1:** Facies of individuals with ASXL3 loss-of-function mutations reported herein demonstrating down-slanting palpebral fissures, a long, tubular nose with a prominent nasal bridge is apparent in most. Most individuals have a broad nasal tip with low columella. The mouth is wide with full (everted) lower lip; hypertelorism, a narrow head shape with prominent forehead, ‘pencilled’ and/or high-arched eyebrows.

**Figure 2.** Map of ASXL3 mutations reported to date. Mutation nomenclature according to HGVS guidelines (<http://varnomen.hgvs.org/>) using NCBI reference Transcript NM\_030632.3).

**Table 1:** Clinical features of twelve previously-unreported patients with ASXL3 loss-of-function mutations reported herein in comparison to previously reported patients with Bainbridge-Ropers syndrome.

**Supplementary section** containing a detailed clinical summary of all patients reported here.



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[illegible]

	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12	R/V
Decipher ID	275029	274593	278695	261513	265854	271912	275860	265908	259240	272591	208772	
ASXL3 Variant	c.1201del p.(Ala401fs)	c.1074T>A p.(Tyr358*)	c.4144C>T p.(Gln1382*)	c.1783C>T p.(Gln595*)	c.3355dup p.(His1119fs)	c.1082dup p.(leu362fs)	c.3635T>G p.(Leu1212*)	c.3127_3128dup p.(Gly1045fs)	c.3178dupC, p.(Arg1060fs)	c.1484insTGAA, p.(Asp497Ter)	c.1491dup p.(Asn498*)	
Age	22yrs	6yrs	6yrs	9yrs	6yrs	10yrs	20yrs	4yrs 3mths	9yrs	19yrs	8yrs	
Sex	F	F	F	M	M	Male	Female	Female	Male	Female	Male	
Height	<0.4 <sup>th</sup>	25 <sup>th</sup>	9 <sup>th</sup>	25 <sup>th</sup>	75 <sup>th</sup> -91 <sup>st</sup>	50 <sup>th</sup>	2 <sup>nd</sup>	50 <sup>th</sup>	25 <sup>th</sup>	9 <sup>th</sup>	50 <sup>th</sup>	
Weight	0.4 <sup>th</sup>	50 <sup>th</sup>	<0.4 <sup>th</sup>	25 <sup>th</sup> -50 <sup>th</sup>	50 <sup>th</sup>	>99 <sup>th</sup>	<0.4 <sup>th</sup>	0.4 <sup>th</sup>	9 <sup>th</sup>	2 <sup>nd</sup>	25 <sup>th</sup>	
OFC	<0.4 <sup>th</sup>	<0.4 <sup>th</sup>	0.4 <sup>th</sup> -2 <sup>nd</sup>	0.4 <sup>th</sup> -2 <sup>nd</sup>	0.4 <sup>th</sup>	<25 <sup>th</sup>	<0.4 <sup>th</sup>	0.4 <sup>th</sup>	<0.4 <sup>th</sup>	0.4 <sup>th</sup>	50 <sup>th</sup>	
Pregnancy	Polyhydramnios	Normal	Normal	Normal	Normal	Normal	IVF (twin well)	Normal	Mild renal pelvis dilatation at 20/40, resolved postnatally	Normal	Normal	11/12
Birth	Em CS; breech; BW- 2.98kg	Em CS; BW- 3.12kg	Normal; BW- 2.7kg	Em CS; breech; BW- 3.5 kg	Induced	Normal	Induced, Em CS	El CS; breech	El CS; extended breech; BW- 3.4kg	Normal; BW- 3.4 kg	El CS; breech	9/12 CS
Neonatal Unit	Yes, 5/7	No	No	No	Yes, apnoea	No	No	No	No	Febrile	No	4/12
Gestation	Term	Term	Term	39/40	42/40	41 weeks	40 weeks	38/40	38/40	40 weeks	38 weeks	
Feeding Difficulties	Yes: Poor suck/swallow, GOR, fundoplication at 3yrs	No	Yes: Hx of GER, feeding issues, poor appetite	Yes: Stopped breast feeding at 4mths due to poor weight gain	Yes: gastrostomy fed; GOR; fundoplication	No	Yes: Severe, NG fed for 2 yrs	Yes	Yes: GOR; FTT	Yes: frequent vomiting as a baby	Yes	9/12
Cranio-facial features	hypertelorism, hirsutism, finely 'pencilled' eyebrows	hypertelorism, down-slanting palpebral fissures	thick eyebrows, prominent forehead, high nasal bridge, deep set eyes	posteriorly rotated ears, dental overcrowding, long jaw	prominent nasal bridge, tongue tie, micrognathia	synophrys, finely 'pencilled' eyebrows, micrognathia, prominent central incisors	scaphocephaly, prominent nasal bridge, mild synophrys, crowded teeth, high palate	-	long face with slightly upslanted palpebral fissures and twin hair crowns	Extremely slim and long limbed	-	9/12
Downslant pf	No	Yes	Yes	No	No	Yes	No	No	No	Yes	Yes	6/12
Palate	High-arched	Narrow, high-arched	NAD	High-arched	High-arched	NAD	High-arched, bifid uvula	Normal	High-arched	High-arched, narrow palate	High-arched	9/12
Strabismus	Yes, divergent	Yes	Yes	No	Yes	No	Bilateral, convergent/divergent	Yes	No	No	Yes	7/12
Hypotonia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	12/12
Marfanoid habitus	No	No	Yes	No	No	No	No	No	-	Yes	No	3/12
Skeletal features	Camptodactyly 4th fingers, short distal phalanx of thumbs, scoliosis	hyperflexible elbows	arachnodactyly	No	No	No	narrow small feet, pes planus	No	postural scoliosis	Malar hypoplasia, long slim hands and feet	No	7/12
ID	Profound	Moderate	Severe	Severe	Severe	Moderate/Severe	Severe	Moderate	-	Severe	Severe	12/12
Seizures	Yes; absences	No	No	No	No	No	Possible absences 6y; GTCS from 11y	No	No	GTCS in childhood	Yes	3/12
Autism/ ASD	Likely; not formal	No	ASD	Likely (no formal Dx)	No	Autism	Autism (Dx 6yrs)	No	ASD, SDP, ADD	Likely (no formal Dx)	Yes	9/12
Structural anomalies	unilateral choanal stenosis	No	No	No	No	No	Bifid nail, laryngomalacia, intestinal malrotation	No	No	No	No	2/12
Speech and language	only noises	v. delayed	v delayed	makes noises and uses Makaton	Communicates by looking at objects	Non-verbal, displays comprehension	Only simple sounds, no words: uses PECS	delayed	Uses PECS	-	no expressive language	12/12
Behaviour	Autistic traits, hyperventilates	No	autistic traits	No	Periodic agitation with self-harming	Poor sleep; difficult behaviour	Frustrated; hand flapping when upset	-	Pica; sleep disorder	Grunting; bruxism	generally placid	9/12
Hand flapping	Yes	No	No	No	No	No	Yes	shakes and claps hands	head rocking and clicking	-	Yes	3/12
Educational support	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: special school	Yes: SEN	Yes: SEN	Yes: special school	12/12

M: Male; F: Female; NR: Not reported; NA: Not applicable; PD: Palpebral fissures; ASD: Autism spectrum disorder; ID: Intellectual disability; Dx: Diagnosis; NAD: No abnormality detected; BW: Birth weight; Em CS: Emergency caesarean section; El CS: Elective caesarean section; SEN: Special educational needs; GOR: gastro-oesophageal reflux; FTT: Failure to thrive; GTCS: generalised tonic-clonic seizures; PECS: Picture exchange communication system; -: Not known; R/V: review summary

**SUPPLEMENTARY MATERIAL****Clinical Description**

**Patient 1:** This 21-year old patient was the first child of healthy, non-consanguineous, White European parents. He has a younger sibling who is fit and well. The family history was complicated by paternal history of TIA's (transient ischaemic attacks), although MRI-brain scan in the father was reported as normal. He was conceived naturally and there were no concerns during the pregnancy. He was born full-term with a birth weight of 3699 grams by elective cesarean section due to breech presentation. He was initially admitted to the Neonatal unit due to a lung collapse needing chest drain insertion and ventilatory support for 5 days. He was discharged home at 11 days of age and there were no reported feeding difficulties.

Concerns were initially identified when his developmental milestones were delayed: he smiled at 20-weeks of age, sat up at 12-months of age and walked at 23-months of age. His speech was particularly delayed in that his first words were at 18-months of age and he needed speech therapy. He was also diagnosed with an Autistic spectrum disorder (ASD) and went on to attend a special needs school. He was subsequently diagnosed with Tourette's syndrome, needing treatment with Aripiprazole. A MRI-brain scan was reported as grossly normal with minor terminal myelination defects. There were no concerns with his general health and although, he had 2 episodes of febrile seizures as a child, he does not suffer from any seizures.

On examination, he had the following dysmorphic facial features: relatively long, tubular nose, thin upper lip, down-slanting palpebral fissures (Figure A). Growth parameters at the age of 15-years were: height 172.2 cm (75<sup>th</sup> centile), weight 60.3 kg (75<sup>th</sup> centile), and ofc 54.6 cm (9<sup>th</sup>-25<sup>th</sup> centiles). His lower limb reflexes were brisk with down-going plantar responses but the rest of his neurological examination was unremarkable. Previous investigations have included: normal 60K Array; normal Fragile-X and GCH1 testing.

**Patient 2:** This 22-year old patient was the second of 5 children born to healthy, non-consanguineous White European parents. There was no relevant family history. She was conceived naturally. The pregnancy was complicated by polyhydramnios. She was delivered at 38 weeks gestation by elective caesarean section for breech presentation , with a birth weight of 2980 grams. She was admitted to the neonatal unit because of poor feeding. She was noted to have a unilateral choanal stenosis and a small jaw. She was breast fed initially but failed to thrive and was switched to bottle feeding with added polycal.

Concerns were raised within the first 3 months about lack of alertness, reduced visual behaviour and hypotonia. She had severe gastro-oesophageal reflux requiring fundoplication at 3 years and a gastrostomy primarily to vent swallowed air. She sat at 9 months but has never been ambulant but will weight bear with support. She can reach and handle things with both hands and can press switches. She can feed herself with hands or spoon and enjoys food. Vocalisation is limited to noises and clicks. She is able to use picture exchange to choose between 2 options. Understanding is very limited and is situational. There is very poor eye contact and gaze avoidance and she will explore objects non-visually. She does enjoy observing others. Hearing appears normal. She displays head rolling, thumb twiddling, bruxism, mouthing and eye pressing behaviours.

Menarche was at 10 years but menstruation has been infrequent. She required surgical rodding for scoliosis. Absence seizures and occasional partial seizures are treated with Sodium Valproate.

On examination, she had hypertelorism with marked divergent squint, finely pencilled eyebrows, high-arched palate and hirsutism (Figure B). She had contractures of the 4<sup>th</sup> fingers with shortened distal phalanx of the thumbs and out-turning of the feet. She tended to adopt a head-tilted posture. Growth parameters at 19 years were height 125cm (<<0.4<sup>th</sup> centile) weight 41kg (<0.4<sup>th</sup> centile) and ofc 51.5cm (<0.4<sup>th</sup> centile).

Previous investigation have included: normal 60K array, normal molecular testing of MECP2, cranial CT scan.

**Patient 3:** This 6-year old patient was the second child of healthy, non-consanguineous White European parents. She has an older sibling who has severe cerebral palsy. There is nothing else of significance in the rest of the family history. She was conceived naturally and there were no concerns during the pregnancy. She was delivered by planned caesarean section at 39 weeks gestation with a birth weight of 3118 grams.

Concerns were initially identified when her developmental milestones were delayed: she sat up at approximately twelve months; had previously rolled at around the age of nine months. At the age of eighteen months, she was pulling to stand and cruising around the furniture. She walked independently at 3 years. She was toilet-trained at 4 years. She had no words until 5 years, but said “dada” from around 12 months. She has relatively good social communication

skills despite her speech difficulty. She wears glasses for hypermetropia and has a mild left convergent squint. There are no concerns with her general health.

On examination, she was dysmorphic with hypertelorism, down-slanting palpebral fissures (Figure C), her height at 18 months was between the 25th and 50th centiles, her weight between the 9th and 25th centiles and ofc between the 0.4th and 2nd centiles, at 6 years her height and weight were on 25th centile and ofc just below 0.4th centile. Parental head circumferences are both on the 75th centile.

Previous investigations have included: chromosomes, 15q methylation, SMN testing, full metabolic work-up all of which were normal.

**Patient 4:** This 6-year old patient was the third child of healthy, consanguineous Libyan parents. There was no relevant family history. She was conceived naturally and the pregnancy was normal. She was delivered at term, with a birth weight of 2700 grams. There were no neonatal concerns other than gastro-oesophageal reflux.

Concerns were raised about her motor development which was mildly delayed: she sat at 9 months and walked at 20 months. However, she had significant speech delay and did not progress from the babble stage. When assessed at 6 years, she still did not have any formal speech and her understanding of language was also very limited. Her behavioural profile was of particular concern: she showed poor social interaction and communication; she had no sense of danger; she had a short attention span and was easily distractible; she required Melatonin to aid sleep. She had a number of repetitive mannerisms such as shaking her head from side to side. Aged 4.5 years, she underwent a formal behavioural assessment which



confirmed a diagnosis of Autism Spectrum Disorder. She had a divergent squint but otherwise her vision and hearing were normal. Her general health was good other than poor weight gain.

On examination, she had a thick head of hair and thick eyebrows. She had a prominent forehead with a high nasal bridge and deep set eyes (Figure D). She had a mild pectus deformity but no scoliosis. She was of very slim build with slender hands and feet. Growth parameters at 6 years were height 109.8cm (9<sup>th</sup> centile), weight 13.5kg (<0.4<sup>th</sup> centile) and OFC 48.8cm (0.4<sup>th</sup> centile).

Previous investigations included microarray which showed a paternally inherited 3.2 Mb gain at chromosome 4p15.1 which contained no known dosage sensitive genes and so was thought to be of unlikely clinical significance. Neurometabolic investigations were also completed and were normal. MRI-brain scan performed aged 3.5years showed minor periventricular high signal of uncertain significance.

**Patient 5:** This 9-year old patient was born following a normal pregnancy, breech presentation was diagnosed. He was delivered by elective Caesarean section. His unaffected brother had also been a breech presentation. His birth weight was 3500 grams and he was in good condition. There were no concerns with feeding or growth at first but over the first few months, feeding deteriorated and he failed to thrive. At four months of age, breast feeding was stopped and he was given formula feeds with calorie supplementation. At 6 months of age, he had an episode of bronchiolitis associated with significant weight loss. He recovered from this and there were no problems with weaning to solid food.

In terms of his development, he developed a social smile at 6 weeks of age but all other milestones were delayed. He had low muscle tone, sat late and walked independently at approximately 4 years of age. He has no recognisable words but makes sounds and communicates using Makaton. He has not had any seizures. He does not demonstrate hand flapping. He does periodically have tics or habits which resolve after a time. He has not been formally assessed for autism. He is generally a sociable boy.

On examination at 9-years of age, his growth parameters were, OFC 51.7 cm (2<sup>nd</sup> centile), height 128.0 cm (9<sup>th</sup> centile), and weight 26.0 kg (9<sup>th</sup> centile). He has a narrow head shape with a prominent forehead but no overt scaphocephaly and his cranial sutures are normal. He has a high-arched palate with dental overcrowding and a long jaw (Figure E). Physical examination was otherwise unremarkable.

**Patient 6:** This 6-year old boy was born at 42-weeks gestation following a normal pregnancy by normal delivery. He was found to have micrognathia with a high-arched palate, a prominent nasal bridge and long, slender fingers and toes (Figure F). He had a prominent metopic ridge but a skull X-ray showed normal sutures. In the first few days of life, he developed feeding difficulties associated with palatal-pharyngeal incoordination. Severe reflux and feeding difficulties necessitated fundoplication and gastrostomy. He had truncal and limb hypotonia. At 6 years of age, cyclical periods of agitation developed which were subsequently found to be due to Crohn's disease.

In terms of his development, he smiled at an early age but all subsequent development was delayed. He sat independently at approximately 6 years of age. At 9 years of age, he can stand

with support, is not walking, vocalises a little but with no words but has some understanding. He has long, slender limbs.

Microarray was normal. FISH studies on skin fibroblasts excluded trisomy 8 mosaicism. A CT-brain was normal.

**Patient 7:** This 10-year old patient was born at 41 weeks following an unremarkable pregnancy. Birthweight was 3090 grams and there were no initial concerns. Concerns were first raised at the age of 10 months with a suggestion of motor delay, and at that point a diagnosis of dystonic cerebral palsy was made. Since then patient has shown global developmental delay, sitting at 10 months, but not walking until after the age of 5. He remains non-verbal, communicating with Makaton, but clearly has some understanding of language. He has a formal diagnosis of autism spectrum disorder with his developmental delay. He has additional behavioural problems and poor sleep. Height and head circumference have remained in the normal range, but his weight is persistently on or greater than the 99th centile for age.

On examination, he has dysmorphic facial features with synophrys and finely ‘pencilled’ eyebrows (Figure G). His palpebral fissures are upslanting. He has a thin lower lip, micrognathia and strikingly prominent central incisors. ArrayCGH and other baseline investigations for learning difficulties were normal.

**Patient 8:** This 19-year old female patient is one of twins, the only children of White Welsh parents. The patient's twin brother is healthy with no intellectual problems. There is no family history of note. The twins were conceived by IVF. The pregnancy was uncomplicated. The

patient was born by emergency caesarean section (for failure to progress) at 40 weeks gestation. APGAR scores were good. Birth weight was 3090g (25th centile) and head circumference was 34.8cm (50th centile).

The patient had early severe feeding problems and was nasogastric tube fed from day one. She had recurrent admissions for chest infections in the first year of life and was diagnosed with laryngomalacia and gastro-oesophageal reflux. She was reviewed in the Genetics Clinic at 5 months of age she was noted to have mild dysmorphic features (mild synophrys, cupped ear helix, down turned mouth), microcephaly, hypotonia and poor weight gain. The patient had an operation for intestinal malrotation at 9 months of age. Nasogastric feeding continued for 1-2 years.

This patient was noted to have global developmental delay in infancy. She sat at 18 months and walked at 8 years. She was diagnosed with autism at 6 years. She was taught with 1:1 help in a special school. The patient had possible absence seizures from 6 years but developed generalised tonic-clonic seizures from 11 years. Her periods began at 12 years.

On examination at 19 years of age, her height was 152cm (2nd centile), weight 37.3kg (<0.4th centile) and head circumference 50.4cm (<0.4th centile) and dysmorphic with down-slanting palpebral fissures, hypertelorism, and prominent forehead (Figure H). She had mild synophrys with lateral upswing of the eyebrow, prominent nasal bridge and root, a small mouth with short philtrum, high palate, bifid uvula, crowded teeth, an overbite, a convergent squint, slender feet, pes planus, scaphocephaly and slightly low-set ears. She can walk short distances. She has no speech apart from simple sounds but uses a PECS (Picture Exchange Communication system) effectively. She uses a spoon to feed herself. Her neurology was

difficult to assess but she appears to have low muscle tone. The patient has a bifid nail on her left index finger.

Potential diagnoses suggested over the years include: Angelman syndrome, Pitt-Hopkins syndrome and Cornelia de Lange syndrome. Extensive metabolic investigation was normal. MRI-brain at 1 year showed delayed myelination. This was normal when repeated at 9 years. Genetic tests have included: routine karyotype (blood and skin), subtelomeric screening, Angelman methylation, TCF4 and array CGH – all normal.

**Patient 9:** This 4-year old patient was born following a normal pregnancy at 38-weeks gestation by elective caesarean section due to breech presentation with a birth weight of 3200 grams. She was in a good condition immediately after birth and did not need admission to the Neonatal unit. She has feeding difficulties in the first year of life which subsequently resolved. She went on to have moderate developmental delay with walking at over 1 year of age, speech delay and need for special school.

This patient was initially reviewed in the Genetics clinic at 2-years of age. On examination at 4 years of age, her height was 97 cms (50<sup>th</sup> centile), weight 12.05 kg (0.4<sup>th</sup> centile) and ofc 47.5 cm (0.4<sup>th</sup> centile); she had hand flapping behaviour with severe speech delay. She had distinctive facial features including down-slanting palpebral fissures, fine arched eyebrows, prominent forehead, bilateral low-set ears (Figure I).

**Patient 10:** This 9-year old patient was antenatally diagnosed with mild renal pelvis dilatation which subsequently resolved. He was born by elective caesarean section due to extended breech presentation at 38-weeks gestation with a birth weight of 3400 grams. He was in a

good condition immediately after birth and did not need admission to the Neonatal unit.

However, he developed significant gastro-oesophageal reflux and failure-to-thrive following cessation of breast feeds and had significant feeding issues between 6-8 months of life.

In terms of his development, he was delayed in that he had marked central hypotonia with significant head lag persisting until 3 years of age; sat up at 8-months but delayed with his walking with unusual gait and dystonic posturing of lower limbs; poor speech relying on PECS communication. He currently attends a special needs school and has been diagnosed with autism spectrum disorder, attention deficit with previous trial of Methylphenidate and Clonidine for stereotypies. In the first couple of years of life, he had frequent colds and cold extremities. He has a pleasant disposition with broad smile but has obsessive behaviours and high pain threshold.

On examination at 7 years, 7 months of age, his height was 120 cm (25<sup>th</sup> centile), weight was 19.4 kg (9<sup>th</sup> centile) and ofc 50 cm (<0.4<sup>th</sup> centile) with distinctive facial features including up-slanting palpebral fissures, twin hair crowns, high-arched palate and bilateral low-set ears. He also had a postural scoliosis needing Lycra suit fitting. MRI-brain and arrayCGH were reported as normal

**Patient 11:** This 19-year old patient was born following a normal pregnancy at 40-weeks gestation with a birth weight of 3400 grams by normal delivery. She developed a febrile episode on day 5 needing neonatal admission. She also had significant feeding difficulties with frequent vomiting episodes.

In terms of her development, she was delayed in that she had marked central hypotonia with head lag, she sat independently at 2 years 4 months and walked at 5 years with an unsteady gait. She is non-verbal and attends a special needs school. Her behaviour is complicated by bruxism, grunting, mouthing of hands, intermittent eye contact. She also developed generalised seizures as a child which have now resolved.

On examination at 11.5 years of age, her height was 136.4 cm (9<sup>th</sup> centile), weight was 26.3 kg (2<sup>nd</sup> centile) and ofc 52 cm (0.4<sup>th</sup> centile), she is extremely slim with long limbs, arachnodactyly, malar hypoplasia, a high-arched narrow palate and down-slanting palpebral fissures.

**Patient 12:** This 8-year old patient was born following a normal pregnancy at 38-weeks gestation by elective caesarean section due to breech presentation. He was in a good condition immediately after birth but went on to have mild feeding difficulties not needing any intervention.

In terms of his development, he was delayed in that he developed social smile at 3-months of age, sat independently at 18-months of age, walked with support at 3 years and independently at 5 years of age but has no speech. He attends a special needs school and is said to be generally placid with a diagnosis of autism spectrum disorder.

On examination at 8 years of age, his height and ofc were on the 50<sup>th</sup> centile and weight on the 25<sup>th</sup> centile with a high-arched palate, down-slanting palpebral fissures and hypotonia (Figure J).